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Antioxidant properties of *Rosa pisiformis* and its protective effect against isoproterenol-induced oxidative stress in rats

Rosa pisiformis'in antioksidant özellikleri ve izoproterenol ile ratlarda oluşturulan okdidatif strese karşı koruyucu etkisi

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Abstract: Objective: In this study, *Rosa pisiformis* was evaluated for its antioxidant activity, vitamin (A, E, C), trace element (Fe, Cu, Zn, Mn, Cr, Se) and mineral (Ca, K, Mg, Na) levels, in addition to its cardioprotective effects on cardiac tissue antioxidant enzymes (GSH-Px, SOD, CAT) during isoproterenol (ISO) - induced oxidative stress.

Methods: In the study, vitamin and mineral analysis was carried out using HPLC and ICP-MS method, *R. P.* antioxidant and antiradical properties were determined using spectrophotometer. Forty wistar albino rats were divided into four groups: control (0.9% NaCl), ISO (100 mg/kg), ISO (100 mg/kg) + *R. P.* (300 mg/kg) and *R. P.* (300 mg/kg). ISO was injected subcutaneously into rats twice at an interval of 24 h for two consecutive days (on 28th and 29th day). *R. P.* fruit administered daily. The experimental period was continued for 30 days.

Results: The results of the study indicated that *R. P.* fruit and leaves contain high levels of minerals and vitamins and possess antioxidant and antiradical activity and its fruits have positive effects on cardiac tissues. The results are supported with significant changes in antioxidant enzyme levels and histopathological examination.

Conclusion: This study demonstrates that *R. P.* fruits in particular can be used to treat and prevent cardiac diseases.

Keywords: *Rosa pisiformis*, Isoproterenol, Trace element, Vitamin, Antioxidant enzymes

Özet: Amaç: Bu çalışmada *Rosa pisiformis*'in antioksidant aktivite, vitamin (A,E,C), iz element (Fe, Cu, Zn, Mn, Cr, Se) ve mineral (Ca, K, Mg, Na) düzeyleri, bununla birlikte, isoproterenol ile oluşturulan oksidatif stres boyunca, kalp dokusu antioksidan enzimleri (GSH-Px, SOD, CAT) üzerine koruyucu etkileri değerlendirildi.

Metod: Çalışmada, vitamin ve mineral analizi HPLC ve ICP-MS metodu kullanılarak gerçekleştirildi. *R. P.* antioksidant ve antiradikal özellikleri spektrofotometre kullanılarak belirlendi. Kırk wistar albino rat dört guba ayrıldı: Kontrol (0.9% NaCl), ISO (100 mg/kg), ISO (100 mg/kg) + *R. P.* (300 mg/kg) ve *R. P.* (300 mg/kg). ISO ratlara 24 saat ara ile 2 gün (28 ve 29. gün) boyunca iki kez subkutan olarak enjekte edildi. *R. P.* meyvesi günlük olarak uygulandı. Deneysel aşama 30 gün sürdürüldü.

Bulgular: Bu çalışmanın sonuçları, *R. P.* meyve ve yapraklarının yüksek düzeylerde mineral ve vitamin içeriği, antioksidan ve antiradikal aktiviteye sahip olduğu ve

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meyvelerinin kalp dokusu üzerinde olumlu etkilere sahip olduğunu gösterdi. Bu sonuçlar, antioksidan enzim düzeylerinde ve histopatolojik incelemedeki anlamlı değişiklikler ile desteklendi.

Sonuç: Bu çalışma, *R. P.* meyvesinin özellikle kalp hastalıklarının önlenmesi ve tedavisi için kullanılabileceğini göstermektedir.

Anahtar Kelimeler: *Rosa pisiformis*, İzoproterenol, İz element, Vitamin, Antioksidan enzimler

1 Introduction

Isoproterenol is a synthetic catecholamine, which causes severe stress in myocardial tissue, and its high doses produce acute myocardial necrosis. Free radicals produced by ISO treatment reportedly initiate membrane-bound polyunsaturated fatty acid peroxidation, leading to both functional and structural myocardial injury [1].

The antioxidant defense systems including enzyme (superoxide dismutase, catalase and glutathione peroxidase) and non-enzyme defenses (glutathione, vitamins C and E) play an important role in scavenging oxidants and preventing cell injury [2]. SOD is a class of enzymes that catalyzes the dismutation of two superoxide radicals to form hydrogen peroxide and molecular oxygen. Thus, generated H_2O_2 is inactivated by either catalase or by the GSH redox system consisting of reduced glutathione as the cofactor for GSH-Px and glutathione reductase [3].

Vitamin A (retinol) and vitamin E (α-tocopherol) are lipid-soluble vitamins that are essential for human health. Both groups have free-radical scavenging properties, which allow them to function as physiologic antioxidants in protecting several chronic diseases, such as cancer and cardiovascular disease [4]. Vitamin C is an important water-soluble antioxidant. It protects compounds in extracellular and intracellular spaces in most biological systems and reduces tocopherol radicals back to their active form at cellular membranes. It can directly scavenge superoxide radicals, molecules of singlet oxygen, hydrogen peroxides and hydroxyl radicals [5].

Trace elements are particularly essential for all plants. Most trace elements are reportedly necessary for a few species and the rest have a stimulant effect on plants. Trace elements have toxic effects on cells in high concentrations and play a key role in metabolic pathways, such as respiration and photosynthesis. It is stated that most trace elements (copper, aluminum, cobalt, molybdenum, manganese, and zinc) are involved in the protective and defense mechanisms in some plant species [6,7].

In recent years, cancer and cardiovascular disease prevention has been associated with the ingestion of fresh fruits, vegetables or teas that are rich in natural antioxidants [8]. Among the members of Rosa L. within the family Rosacae, rose hips have gained much interest for therapeutic studies. Because they are abundant in nature and antioxidant compounds, such as bioflavonoids, organic acids, vitamins, minerals, and carotenoids, they are becoming popular for therapeutic purposes and are the subject of our studies.

This study aimed to investigate the vitamin and mineral content, total phenolic and total flavonoid content, antioxidant capacity, and the free radical-scavenging activity of the plant *Rosa pisiformis* (Christ). D. Sosn., which is used against cardiovascular diseases and is one of the endemic taxa of the rose hip species. Its protective effect on the antioxidant defensive system in rats subjected to catecholamine ISO-mediated oxidative heart injury was also investigated.

2 Materials and Methods

2.1 Plant material and extraction

Rosa pisiformis (Christ.) D. Sosn. leaves and fruits were collected in August 2011 in the Hosap Güzelsu district, Van, bahceci village. A specimen was deposited in the Department of Botany Herbarium, Yüzüncü Yıl University, VANF F13827 END.

2.2 Extraction

Rosa pisiformis (Christ.) D. Sosn. leaf and fruit methanol extraction was performed according to the method of Cai *et al.* [9], with slight modifications. The ground samples were dried at room temperature (23°C). Samples were ground to a fine powder using a Kenwood Multi-Mill (Kenwood Ltd., UK) and passed through a sieve (24 mesh). In total, 5 g sample was extracted with 100 mL 80% methanol at 35°C for 24 h. The samples were then cooled to room temperature, centrifuged at 5,000 rpm for 10 min and filtered by Whatman No. 1 paper. Extracts were evaporated to dryness in a vacuum and lyophilized. Methanol extracts were placed in a dark bottle and stored at -20°C until used.

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2.3 *In vitro* antioxidant activity studies

2.3.1 Total antioxidant activity determination

Total antioxidant activities in *R. P.* leaf and fruit methanol extracts were evaluated using the method described by Prieto *et al.* [10], Briefly, 0.2 mL sample was mixed with 2 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a boiling water bath at 95°C for 90 min. The samples were then cooled at room temperature, and the absorbance was measured at 695 nm against the blank in a spectrophotometer. Total antioxidant activity is expressed as the number of ascorbic acid equivalents in mM per gram extract.

2.3.2 DPPH radical scavenging activity

DPPH assay uses stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a reagent Chen *et al.* [11], and Cuendet *et al.* [12]. In total, 50 µL extract at various concentrations in methanol were added to 5 mL 0.004% DPPH methanol solution. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Sample antioxidant activity was expressed as IC_{50} values (µg/mL) required to inhibit DPPH radical formation by 50%, which was calculated from inhibition plot. IC_{50} values are expressed as the means±SD of triplicate experiments. The synthetic antioxidant butylated hydroxytoluene was included in experiments as a positive control.
Inhibition of free radical DPPH in percent was calculated using the following formula:

$$\text{Inhibition}\,(\%) = \left\{\frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}}\right\} x\,100$$

Where ABlank was the absorbance of the blank sample and Asample was the absorbance in the presence of the *R. P.* leaf and fruit methanol extracts.

2.3.3 Superoxide radical scavenging activity

The *Rosa pisiformis* leaf and fruit extract superoxide radical scavenging activity was determined according to the method of Robak and Gyglewski [13], and Lee *et al.* [14], with some modifications. The solution was prepared by dissolving xanthine (2 nM) and NBT (12 nM) in 0.1 M potassium phosphate buffer (pH 7.4). The mixture was then incubated at 37°C for 10 min. The blue color formed was measured at 560 nm using a spectrophotometer. The superoxide anion radical scavenging activity was calculated using the formula given below:

$$\text{Superoxide anion radical scavenging activity}\,(\%) = \left\{\frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}}\right\} x\,100$$

Sample superoxide radical scavenging activity was expressed as IC_{50} values (µg/mL) required to inhibit superoxide radical formation by 50%), which was calculated inhibition plot. The IC_{50} values are expressed as the means±SD of triplicate experiments. The synthetic antioxidant butylated hydroxytoluene was included in the experiments as a positive control.

2.3.4 Scavenging of hydroxyl radical

Hydroxyl radical scavenging of *Rosa pisiformis* leaf and fruit extracts were measured using the method modified by Kunchandy and Rao *et al.* [15]. The reactive mixture consisted of 1 mM ferric chloride, 3 mM deoxyribose in 20 mM phosphate buffer (pH 7.4), 1 mM EDTA solution, and 20 mM H_2O_2 with or without each extract solution. The reaction was incubated for 60 min at 37°C. A solution of 1% TBA in 1 ml 2.8% TCA was added to the mixture and incubated for 30 min in a boiling water bath. After cooling, the absorbance was recorded at 532 nm. Hydroxyl radical scavenging was calculated as:

$$\text{Inhibition}\,(\%) = \left\{\frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}}\right\} x\,100$$

Sample hydroxyl radical scavenging results were expressed as IC_{50} values (µg/mL) required to inhibit hydroxyl radical formation by 50%), which were calculated from the graph plotted inhibition percentage against extract concentration. The IC_{50} values are expressed as the means±SD of triplicate experiments. The synthetic antioxidant butylated hydroxytoluene was included in the experiments as a positive control.

2.3.5 Total phenolic assay

Total phenolic content in *Rosa pisiformis* leaf and fruit methanol extracts was measured using the modified Folin–Ciocalteu method of Singleton *et al.* [16]. Total soluble phenolic constituents of the *R. P.* methanol extracts were determined by employing methods involving Folin-Ciocalteu reagent and gallic acid as standard. In total, 0.1 mL extract solution containing 1000 µg extract was obtained in a calibrated flask, 45 mL distilled water and 1 mL folin ciocalteu reagent was added, and flask was shaken thoroughly. After 3 min, 3 mL Na_2CO_3 solution (2%, w/v) was added, and mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. Gamez-Meza *et al.* [17], and Yi *et al.* [18]. The same method was repeated with all of the standard gallic acid solutions (25–250 µg/mL). Phenolic

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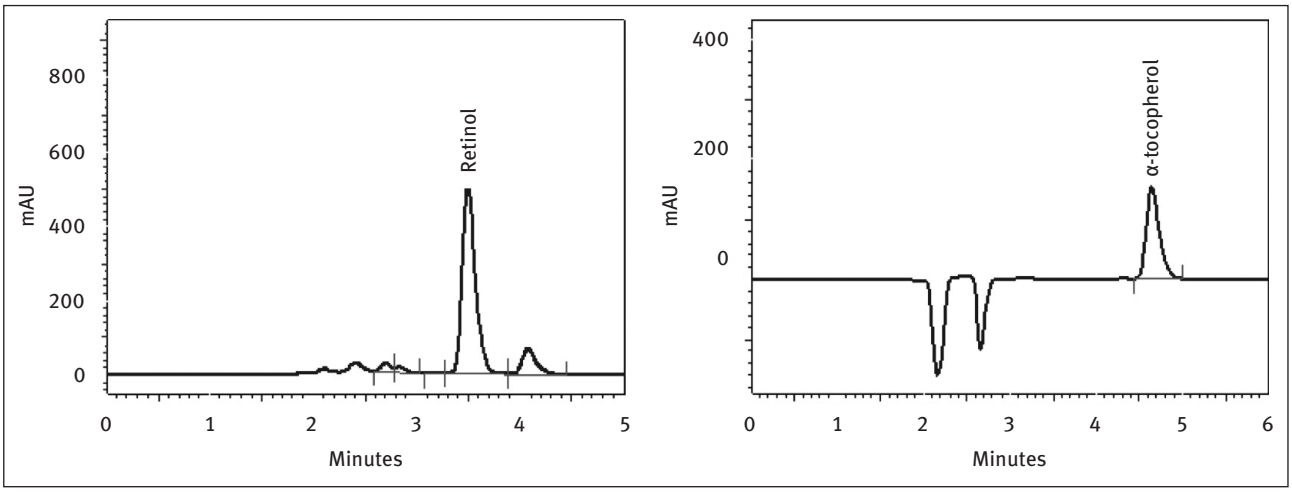


Figure 1: Isocratic elution was performed with methanol-THF (80:20, v/v) as mobile phase at a flow rate of 1.5 mL/min, 40°C. C18 reversed phase column (250×4.6 mm ID) was employed. The chromatogram was monitored with photodiode array detector (PDA) array detection at 325 and 290 nm α-tocopherol and all-trans-retinol.

contents are expressed as gallic acid equivalents per gram (mg gallic acid g-) extract. GA values are expressed as the means±SD of triplicate experiments.

Total phenolic compound content in plant methanol extracts in gallic acid equivalents (GA) was calculated using the following formula:

$$C = \frac{c \times V}{m}$$

Where: C=total phenolic compound content mg/g plant extract) in GA; c=concentration of gallic acid established from the calibration curve (mg/mL); V=extract volume (mL); m=pure plant methanol extract weight (g).

2.3.6 Total flavonoids

Total flavonoid content was determined using an aluminum chloride colorimetric method with quercetin as a standard and expressing the results as mg quercetin equivalents to extract. Briefly, 1 mL 2% aluminum trichloride ($AlCl_3$) in methanol was mixed with the same volume of methanol extracts of *R. pisiformis* leaf and fruit. Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of 1 mL extract solution with 1 mL methanol without AlCl3. Lamasion *et al*. [19], Urgeova and Polivka *et al*. [20].

2.3.7 Trace element and mineral analysis

For the elemental (Cu, Zn, Se, Cr, Co) analysis, ICP-MS (Agilent 7500a USA) was used. Ca, K, Mg, Na, Fe and Mn concentrations were detected by atomic absorption spectrophotometry (Perkin Elmer A Analyst 800).

2.4 Vitamin (A, E) analysis

2.4.1 Standard solutions and calibration

α-tocopherol and all-trans-retinol stock solutions were prepared at 500 μg/mL in methanol (Fig 1). Vitamin C stock solution was prepared at 4000 μg/mL in metaphosphoric acid. Stock solutions were appropriately diluted with the mobile phase for standard solution preparation. Calibration was calculated by linear regression analysis of the peak area versus the standard solution concentrations.

2.4.2 Extraction process

The plant (5 g) was extracted by hexane and ethyl alcohol (containing 0.01% BHT), and the sample was vortexed for 1 min Su *et al*. [21]. The sample was extracted for 30 h in darkness. The sample was vortexed and centrifuged at 5000 rpm for 12 min. The supernatant was filtered using Whatman filter paper, and 500 μL of the hexane layer was extracted and evaporated to dryness under a nitrogen stream at 37°C. The residue was dissolved in THF (50 μL) and methanol was added (150 μL). The sample was vortexed for 1 min then 100 μL. Sample was autosampled using amber glass vials.

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2.4.3 Chromatographic conditions

The chromatographic system consisted of Thermo Scientific Finnigan Surveyor with a photodiode Array detector (PDA) and a tray autosampler (-8°C). The data were processed with Thermo Scientific ChromQuest version 4.2 software. Separation was performed with a 5 µm Gl Science C18 reverse phase column (250×4.6 mm ID). The mobile phase of a methanol-THF mixture (80:20, v/v) was modified from Bruni *et al*. [22]; Al-Saleh *et al*. [4], and Sahin *et al*. [23]. Pump was set at a flow rate of 1.5 mL/min. The chromatogram was monitored with PDA array detection at 325 and 290 nm (α-tocopherol and all-trans-retinol, respectively). The chromatographic analysis was performed at 40°C with isocratic elution.

2.4.4 Vitamin C analysis

The vitamin C method used was modified from the methods of Omaye *et al*. [24]; Golubkina and Prudnik *et al*. [25], and Brewster [26]. Vitamin C content of *R. P.* fruit and leaf were measured spectrophotometrically (UNICAM UV/VIS Spectrometer UV2) at 521 nm according to 2,4-dinitrophenyl hydrazine (DNPH) method.

2.4.5 Experimental animals

In total, 40 male Wistar albino rats (200±50 g weight; 4 weeks old) were used in the study. Rats were obtained from the Animal Care Center, School of Medicine, Yüzüncü Yıl University. Wistar albino rats were housed in a temperature-controlled room (22±2°C) with a 12 h light/dark cycle; water and food were given ad libitum. Experiments were performed according to the guidelines of the animal ethics committee of the Institute. The study protocol was approved by the Yüzüncü Yıl University Animal Experiments Ethic Committee (YUHADYEK, 30.06.2011/Decision no: 06).

2.4.6 Experimental design

The experimental period was performed with 40 male Wistar albino rats. The rats were randomly divided into four groups of ten rats each. Group I: Control 0,9% NaCl, Group II: ISO (100 mg/kg) was dissolved in saline and subcutaneously administered to rats twice at an interval of 24 h for two consecutive days (on 28th and 29th day), Group III: rats were subcutaneously injected with ISO (100 mg/kg) at an interval of 24 h for two consecutive days (on 28th and 29th day) + *R. P.* fruit (300 mg/kg) given by an intra-gastric tube and Group IV: *R. P.* fruit (300 mg/kg) administered orally daily. The experimental period was continued for 30 days. At the end of the experimental period, all the rats were sacrificed; hearts were removed and immediately processed for histopathological studies.

2.4.7 Plant material and extract preparation

Dried *R. P.* fruit (3000 mg) was infused in 300 mL boiled distilled water for 15 min. After decantation and filtration, the filtrate was again dried in an incubator at a 40°C. The aqueous extracts were then obtained daily in isotonic physiological solution (0.9% NaCl). Extract was used to assess cardioprotective properties.

2.5 Antioxidant enzyme activities in heart tissue

2.5.1 Heart tissue preparation

The rats were anesthetized by diethyl ether inhalation and sacrificed. Heart tissues were washed with 0.9% NaCl, tissues were stored at -80°C until analysis. 0.5 g tissue samples were homogenized in 5 mL Ice-cold homogenization buffer (0.32 mol/L sucrose, 1 mmol/L EDTA and 10 nmol/L Tris-HCl, pH 7.4) using a homogenizer (Ultra Turrax T25, IKA, Staufen, Germany) and a glass-porcelain homogenizer (20 KHz frequency ultrasonic, Bandelin Sonupuls) for 5 min and then centrifuged at 9500 rpm for 30 min Xia *et al*. [27]. All of the processes were performed at +4°C, and then the clear upper supernatants were removed for analyses. Supernatants were used to determine antioxidant enzyme activity.

2.5.2 Antioxidant enzyme estimation

The clear supernatant obtained from the heart tissue homogenate was used to assess endogenous antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase. While tissue superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined according to method of Sun *et al*. [28], glutathione peroxidase (GSH-Px) (E.C.1.11.1.9) tissue activity was measured by the method of Paglia and Valentine [29]. Catalase (EC 1.11.1.6) activity was estimated by the method described by Aebi [30]. The results were expressed as IU IU mg/protein (wet tissue weight).

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2.5.3 Total protein concentration determination

Total protein was evaluated by the method of Lowry *et al.* [31]. using bovine serum albumin as a standard. Different concentrations (10–200 µg) of standard protein bovine serum albumin were processed for preparation of a standard curve. The values were expressed as mg protein/g wet tissue (mg/g).

2.5.4 Histopathological analysis of the heart

At the end of the treatments, the rats were sacrificed by decapitation. The hearts were removed, washed immediately with saline, fixed in 10% formalin solution for 24 h and embedded in paraffin. After preparing paraffin blocks, consecutive 5 µm thick sections of the hearts were cut using a microtome and stained with hematoxylin and eosin and Masson's trichrome. The sections were examined under light microscopy (Nikon, Tokyo, Japan) for histological changes.

3 Statistical analysis

Results are expressed as $\overline{X}$±S.D. Analysis of variance (ANOVA) was performed, and the statistical comparisons among the groups were carried out with post-hoc Tukey's test for normally distributed variables, or with nonparametric Benferroni test for non-normally distributed data using a statistical package program (SPSS 22.0 for Windows). Nonlinear regression analysis was used to calculate the IC_{50} values.

4 Results and Discussion

The present study was performed to investigate vitamin (A, E, C), trace element (Fe, Cu, Zn, Mn, Cr, Se) and mineral (Ca, K, Mg, Na) content as well as the *in vitro* antioxidant potential (total antioxidant activity, DPPH, superoxide anion, hydroxyl radical scavenging activity, total phenolic and flavonoid content) of a methanol extract of the leaves and fruit of *R. P.* as well as possible *in vivo* cardioprotective properties against ISO-induced oxidative stress in rats.

Trace elements of the transition metal group activate enzymes or can be incorporated into metalloenzymes of the electron transfer system (Cu, Fe, Mn, and Zn) and can also catalyze substrate valence changes (Cu, Co, Fe, and Mo) [7]. Selenium is a nutritionally essential element for humans and animals because it is a component of selenoproteins, some of which have important enzymatic functions [4].

Table 1: Trace element (Cu, Zn, Mn, Se, Cr, and Fe) and mineral (Ca, K, Mg, Na) content in *Rosa pisiformis* leaf and fruit samples.

Parameters (µg/g)	*Rosa pisiformis* leaves $\overline{X}$±SD	*Rosa pisiformis* fruit $\overline{X}$±SD
Cu	0.46±0.25	9.96±1.37
Zn	27.17±2.34	26.50±2.69
Mn	2.71±0.29	0.90±0.04
Se	0.11±0,08	0.19±0.09
Cr	0.13±0.07	1.41±0.36
Fe	20.38±1.59	1.79±0.24
Ca	455.80±230.57	761.17±190.26
K	256.67±21.66	443.50±22.15
Mg	132.00±75.66	203.17±49.00
Na	4.07±0.17	2.34±0.14

Table 2: Vitamin (E, A, C), total antioxidant activity, total Phenolic and flavonoid content in methanol extracts of *Rosa pisiformis* leaves and fruits.

Parameters	*R. pisiformis* leaves $\overline{X}$±SD	*R. pisiformis* fruit $\overline{X}$±SD
α-tocopherol (µg/g)	7.22±1.37	1.62±0. 37
Retinol (µg/g)	0.43±0.19	0.25±0.06
Vitamin C (µg/100 g)	194.21±9.71	430.69±19.67
Total phenolic content (mg GA/g)	69.72±1.01	20.95±1.13
Total flavonoid content (mg QE/g)	45.09±0.71	20.78±0.83
T. antioxidant capacity (mM A.A/ g)	364.32±1.09	259.36±1.38

4.1 Trace elements, Mineral, Vitamin (E, A, C) and and *in vitro* antioxidant activity

When the mineral levels in the fruits and leaves were compared, Ca, K and Mg content in the fruit was higher than in the leaves, Na content in the leaves was higher than in the fruit, Cu, Cr and Se levels in *R. P.* fruit was higher than in the leaves, and Zn, Fe and Mn content of the leaves was higher than the fruit (Table 1).

The results of our study and similar studies on different rose hip species corroborate the favorable mineral levels. Research has also confirmed that Ca, K, Mg and P content of different rose hip species were generally high, and these species are quite rich in Fe, Mn, zinc and Cu. *R. P.* contains high levels of Ca, Mg, K, Fe, Mn, Zn, Cu and Se; therefore, these data are consistent with other studies [32].

Table 2 demonstrates α-tocopherol, retinol, vitamin C, total antioxidant activity, phenolic and flavonoid content of methanol extracts of *Rosa pisiformis* leaves and fruits.

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Table 3: Percent values of DPPH•+, superoxide anion radical, and hydroxyl radicals in methanol extracts of *Rosa pisiformis* leaves and fruits compared with a positive control.

	DPPH		O_2^-		OH•	
	% Inhib. $\bar{X}$±SD	IC_{50} (µg/mL) $\bar{X}$±SD	% Inhib. $\bar{X}$±SD	IC_{50} (µg/mL) $\bar{X}$±SD	% Inhib. $\bar{X}$±SD	IC_{50} (µg/mL) $\bar{X}$±SD
R. P. fruit	68.34±1.27	45.54±0.51	75.73±1.82	113.19±1.53	65.04±0.62	103.03±3.54
R. P. leaf	82.59±1.08	20.35±1.31	76.36±0.96	70.79±1.45	69.93±0.81	70.75±2.44
BHT	53.68±0.36	59.95±1.85	71.76±1.51	142.95±2.92	60.84±0.84	132.34±9.35

Vitamin A concentrations in *R. P.* fruits was 0.25±0.02 µg/g, and the concentration in the leaves was 0.43±0.05 µg/g. Vitamin E content in the fruits and leaves was 1.62±0.15 µg/g and 7.22±0.69 µg/g, respectively. Vitamin C content was 430.69±11.36 µg/100 g of fruit and 194.21±5.61 µg/100 g of leaves. While evaluating the results, it was observed that *R. P.* fruits and leaves contained vitamins A and E in significant levels.

The present study was performed to evaluate total antioxidant activity of the *R. P.* leaf and fruit methanol extracts. Total antioxidant activity of methanol extracts from the *R. P.* leaves and fruit was 364.32±1.09 mM ascorbic acid/g and 259.36±1.38 mM ascorbic acid/g, respectively. Both *R. P.* extracts exhibited effective and powerful antioxidant activity. These results suggest that the higher levels of antioxidant activity may be due to the presence of phenolic and flavonoid components.

Total antioxidant activity is expressed as the number of ascorbic acid equivalents in mM per gram of extract.
Absorbance ($\lambda_{695\ nm}$)=0.381x[Ascorbic acid (mM)]+0.4448 (r^2: 0.9887)

Total phenolics of various plant extracts were measured using Folin-Ciocalteau's assay while total flavonoids were estimated using an aluminum chloride method for flavonoids. Total phenolic and flavonoid content of plant extracts were determined and expressed in mg gallic acid and quarcetin equivalents, respectively.
Absorbance ($\lambda_{765\ nm}$)=0.0628x[GA (mg)]+0.032 (r^2: 0.9956)
Absorbance ($\lambda_{394\ nm}$)=0.1126x[QE (mg)]+0.7154 (r^2: 0.9565)

Total phenolic and flavonoid content in the present study was evaluated in methanol extracts of *R. P.* leaves and fruit. Phenols are commonly found in plants and reportedly have several biological activities including antioxidant activities. Total phenolic content of *R. P.* leaf and fruit methanol extracts was 69.72±0.59 mg GA/g and 20.95±0.65 mg GA/g, respectively. Flavonoid contents of the *R. P.* leaf and fruit methanol extracts were 45.09±0.41 mg QE/g and 20.78±0.47 mg QE/g, respectively.

A number of studies have investigated the phenolic, flavonoid and antioxidant capacities of various Rosa L. species species Guimaraes *et al.* [33], examined the phenolic and flavonoid content of ripe and unripe fruits and leaves from *Rosa micrantha*, and they determined that the phenolic content was 142.34±3.45 mg GA/g in under ripe fruits, 188.16±21.66 mg GA/g in ripe fruits, and 424.20±31.77 mg GA/g in leaves. The flavonoid content was 12.69±0.50 mg QE/g in under ripe fruit, 19.93±0.81 mg QE/g in ripe fruit, and 78.46±3.92 mg QE/g in the leaves [33]. Phenolic and flavonoid compounds may be responsible for the free radical scavenging activity that was noted. Total phenolic concentration expressed as gallic acid equivalents correlated with the antioxidant activity.

The DPPH, O_2^- and OH• radical scavenging activities of *Rosa pisiformis* methanol extracts along with the standard reference BHT are shown in Table 3.

The sample concentration required to reduce the initial concentration of DPPH by 50% (IC_{50}) under the experimental conditions was determined. A lower IC_{50} value indicated higher antioxidant activity. The best free radical scavenging activity was obtained with the leaf methanol extract (IC_{50} 20.35±0.76 µg/mL), while the fruit methanol extract demonstrated comparable free radical scavenging activity with an IC_{50} value of 45.54±0.29 µg/mL, and the IC_{50} for BHT was 59.95±1.07 µg/mL (Fig 2). It was observed that the leaf methanol extract was more active in scavenging DPPH radicals compared to the fruit methanol extract and the positive control BHT. The fruit methanol extract was more active in scavenging the radicals compared to BHT.

Fenglin *et al.* [34], examined the DPPH radical scavenging activity of leaves, roots and flowers from 300 important medicinal plants including *Rosa cymosa* Tratt. *Rosa banksiae* Ait. and *Rosa chinensis* Jacq. They observed that leaves, roots and flowers were quite active in radical scavenging, and their IC_{50} values were quite low [34].

Another study by Barros *et al.* [35], evaluated the concentrations of ripe and unripe fruits, seeds, leaves and flowers of *Rosa canina* that caused 50% DPPH radical inhibition (IC_{50}). Its fruits, seeds and leaves inhibited DPPH radicals at a low concentration rate of 0.22–3.93 µg/

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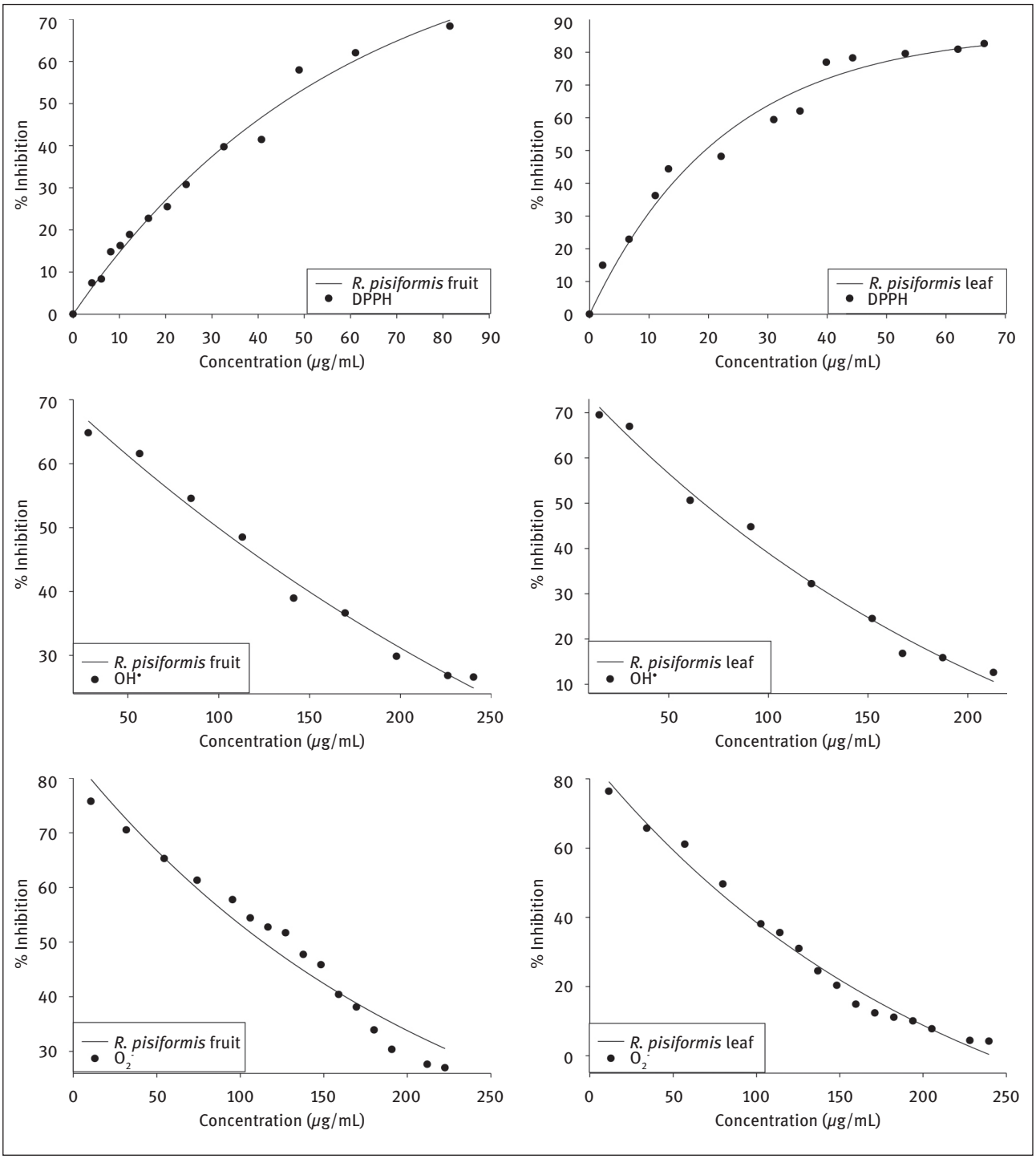


Figure 2: Inhibition of DPPH, $OH^{\bullet}$ and $O_2^{\cdot}$ radicals versus concentrations of *Rosa pisiformis* fruit and leaf methanol extract.

mL [35]. In different study, the concentrations of water and methanol extracts of two different types of rose hips compared methanol extracts of rose hips are reportedly more effective than water extracts [36].

The strongest superoxide anion radical activity was obtained with the leaf methanol extract (IC_{50} 70.79±1.45 μg/mL), while the fruit methanol extract demonstrated comparable levels of superoxide anion radical activity with an IC_{50} value of 113.19±1.53 μg/mL. Thus, the *R. P.* leaf methanol extract demonstrated more potent *in vitro* antioxidant activity with higher percentage inhibition, than the *R. P.* fruit methanol extract (Fig. 2).

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Table 4: Antioxidant enzymes (SOD, GSH-Px and CAT) activities in control, administration with ISO, ISO + *R. pisiformis* and *R. pisiformis* groups in heart tissue samples.

Groups	SOD (IU mg/protein) $\overline{X}$±SD	GSH-Px (IU mg/protein) $\overline{X}$±SD	CAT (IU mg/protein) $\overline{X}$±SD
Control	2842±523.7^{b,b1,c}	31.36±6.39^{a,b}	63.07±8.73^{a,b,c}
ISO	2216±472.2^{c}	16.87±2.93^{a,b1,c}	34.39±3.69^{a,b1,b2}
ISO + *R. pisiformis*	2149±164.1^{b}	23.16±2.26^{b,c}	47.72±3.452^{b,b1}
R. pisiformis	2129±426.3b^{1}	25.15±4.17^{b1}	49.55±12.08^{b2,c}

^{a}: p<0.001; ^{b,b1,b2}: p<0.01; ^{c}: p<0.05 (different letters, significant differences between groups).

It was observed that the fruit and leaf methanol extracts were highly effective in scavenging superoxide radicals. The highest radical-inhibiting rates were 75.73±1.05% for the fruits and 76.36±0.55% for the leaves. The leaf methanol extract have a high radical scavenging effect, demonstrating superoxide radical scavenging activity in lower concentrations than the fruit methanol extract.

The hydroxyl radical scavenging activity was obtained with the leaf methanol extract (IC_{50} 70.75±1.41 µg/mL), while the fruit methanol extract demonstrated comparable superoxide anion radical activity levels with an IC_{50} of 103.03±2.04 µg/mL (Fig 2). Thus, the *Rosa pisiformis* leaf methanol extract demonstrated more potent *in vitro* antioxidant activity with higher percentage inhibition than the *R. P.* fruit methanol extract.

As a consequence of our study, it is show that *R. pisiformis* leaves and fruit are rich in vitamins, total phenol and total flavonoid content; therefore, its antioxidant capacity is quite high. Moreover, it is quite effective in scavenging stable DPPH free radicals and superoxide and hydroxyl radicals; thus, it has anti-radical activity. The antioxidant and other functional properties may be due to the presence of phenolic compounds.

4.2 Activities of antioxidant enzymes in heart tissue

Enzymatic antioxidant activities (superoxide dismutase, glutathione peroxidase and catalase) were studied in controls and different groups that were subjected to *in vivo* ISO-induced oxidative stress. Levels of antioxidant enzyme (SOD, CAT and GSH-Px) activities are presented in Table 4.

Antioxidant enzyme activities were determined in rat heart tissue. Statistical analysis showed that the ISO group was significantly lower than the control group with regards to SOD, GSH-Px and CAT enzyme activities (p<0.05, p<0.001, p<0.001, respectively). The ISO + *R. P.* groups were also significantly lower than the control group regarding SOD, GSH-Px and CAT enzyme activities (p<0.01, p<0.01, p<0.01, respectively). The *R. P.* group was significantly lower than the control group regarding SOD and CAT enzyme activities (p<0.01, p<0.05, respectively), whereas the ISO + *R. P.* group had higher levels of GSH-Px and CAT enzyme activities than the ISO group (p<0.05, p<0.01, respectively). Similarly, *R. P.* group had significantly higher levels of GSH-Px and CAT enzyme activities than the ISO group (p<0.01, p<0.01 respectively). However, no significant increase in myocardial SOD activity was observed in the ISO + *R. P.* and *R. P.* treatment groups, as compared to the ISO group (p>0.05) (Fig. 3).

In the present study, a significant decrease in GSH-Px, SOD and CAT activities was observed following ISO administration. Decreased enzyme activities (GSH-Px, SOD, CAT) in ISO-induced myocardial infarction may be because of increased reactive oxygen species generation, such as superoxide and hydrogen peroxide, which in turn leads to enzyme inhibition [3].

4.3 Histopathological analysis of heart

Heart tissues were stained with hematoxylin and eosin and visualized under light microscopy. Figure 4 illustrated a light micrograph of a histopathological examination of the heart of control, ISO, ISO + *R. P.* and *R. P.*-treated experimental animals.

Histopatologic examination of cardiac tissue sections from the rats in the ISO and *R. P.* fruit group revealed that the negative structural changes observed in the ISO group considerably decreased the degree of myonecrosis and infiltration of inflammatory cells to a lesser extent, and less myocardial injury was observed in their cardiac tissue sections. We examined the cardiac tissue sections of the rats belong to the *R. P.* fruit group.

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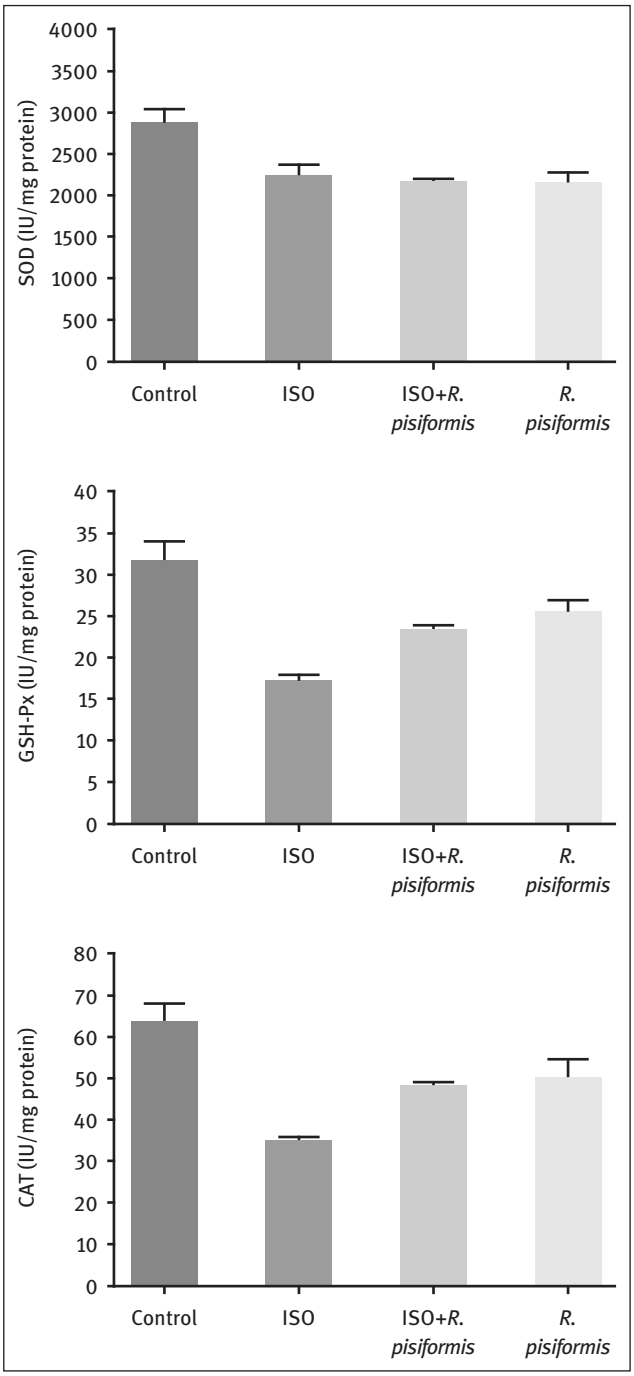


Figure 3: Antioxidant enzyme (SOD, GSH-Px and CAT) activities in cardiac tissue samples of control, ISO, ISO + *R. pisiformis* and *R. pisiformis* groups.

It's observed that myocardium with striated muscle tissue as a normal histologic feature similar to the control group. In general, the histological changes and damage observed in the ISO-administered group were reduced in the ISO and *R. P.* fruit groups. These results indicate that *R. P.* fruits may be effective at preventing ISO-induced cardiac tissue damage.

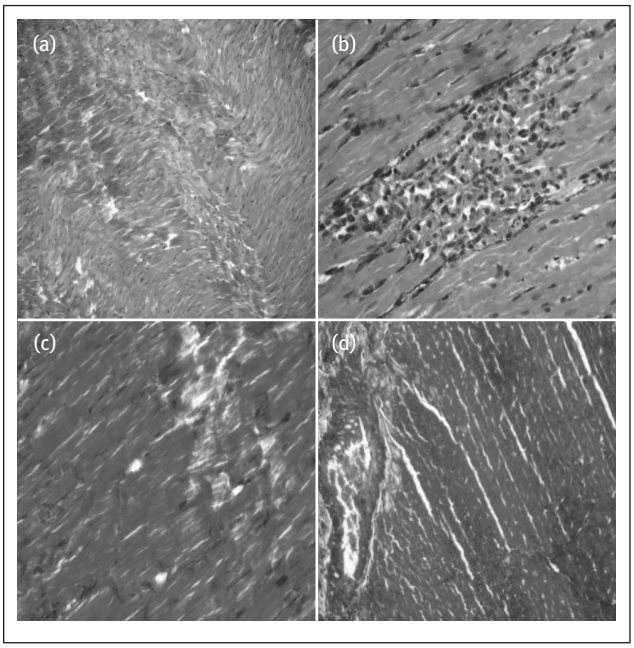


Figure 4: Light micrograph of histopathological examination of the heart of control, ISO and *Rosa pisiformis* treated experimental animals. (A) Control 0.9% NaCl, (B) Isoproterenol (100 mg/kg) (C) ISO (100 mg/kg) + *Rosa pisiformis*. fruit (300 mg/kg) (D) *Rosa pisiformis* fruit (300 mg/kg). Section of heart from Group A showing normal architecture and normal myofibrillar structure with striations H&Ex200. Section of the heart from Group B rats reveals focal myonecrosis and lymphocytic infiltration and chronic inflammation and myophagocytosis H&Ex400. Section of heart from Group C decreased degree of myonecrosis and lesser infiltration of inflammatory cells, less myocardial injury H&Ex400. and Group D rats showing normal architecture of myofibrils with striations H&Ex100.

5 Conclusions

The study clearly demonstrated that *Rosa pisiformis* Christ D. Sosn (*R. P.*) fruit and leaves contain high amounts of minerals and vitamins A, E, C and possess high levels of antioxidant and antiradical activity. *R. P.* leaves could have used as a protective antioxidant in food and pharmaceutical industries. Our results indicated that *R. pisiformis* fruit had a protective effect against oxidative stress. *R. pisiformis* fruit had free radical scavenger properties and could inhibit the generation of ROS, such as DPPH, superoxide anions, and hydroxyl radicals. Its fruits in particular can be used to treat and prevent cardiac diseases. The results are supported with significant changes in antioxidant enzyme levels and histopathological examination.

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